Sclerotinia spp., a potential biological control agent of Canada thistle (Cirsium arvense [L.] Scop.)
by Brenda Simmonds Brosten

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Plant Pathology
Montana State University

Abstract:
In a search for a biological control agent of Canada thistle (Cirsium arvense [L.] Scop.), a survey of endemic pathogens of Canada thistle was conducted in Montana during the summer of 1981. Pathogens from six genera, Alternaria, Fusarium, Septoria, Puccinia, Pseudomonas, and Sclerotinia were obtained. Of these pathogens, Sclerotinia was selected for further study due to its lethality on Canada thistle.

Sclerotinia inoculum for greenhouse or field studies was prepared by culturing isolates of Sclerotinia spp. on autoclaved oat or wheat kernels. The infested kernels were applied fresh or after being air-dried. In greenhouse studies using a kernel inoculation technique, the isolates of Sclerotinia differed in virulence on Canada thistle and also on eleven crops tested. A S. sclerotiorum (Lib.) de Bary isolate was used in all of the field studies.

In field trials conducted on cultivated land infested with Canada thistle, up to 73% of the thistle shoots died within nine weeks of treatment. In addition to attacking the thistle crown and causing wilting and death of the shoots, Sclerotinia infected the roots, extending more than 35 cm into the root system. The damage to the root system reduced the thistle shoot density by 8 to 51% the following year, while the density increased over 40% in the control plots. Soil incorporation of the inoculum reduced the thistle density to lower levels than surface application.

In statewide field trials to test S. sclerotiorum under varied environmental and habitat conditions, 19 to 80% kill of the thistle shoots died within 4 to 8 weeks. The trial site with the highest level of thistle control was in a pasture and, contrary to most of the sites, had not been disturbed by cultivation. At this site, the thistle density in the treated plots was reduced 95% the following year.

Additional research into the host range and genetics of Sclerotinia would be necessary in the development of this fungus as a biological control agent of Canada thistle.

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APPROVAL

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Brenda Simmonds Brosten

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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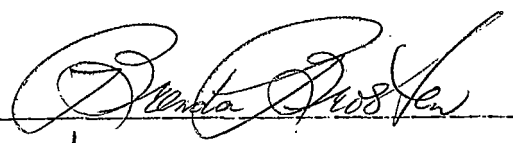
Signature 

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ABSTRACT

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INTRODUCTION

Canada thistle is a serious problem weed infesting range and cropland in Montana and the surrounding states. This noxious perennial weed is estimated to infest over 600,000 hectares in Montana, 680,000 hectares of wheat in North Dakota and 35% of all cultivated fields in Alberta (34,54,73). In addition to rangeland, pasture, dryland crops, and irrigated crops, Canada thistle can also be found in home gardens, along roadways and ditches. Its windblown seeds allow the weed to spread and its extensive perennial root system makes it difficult to control.

The best current control practices include the use of herbicides, intensive summer cultivation, and competitive crops such as alfalfa or perennial forage grasses. However, herbicides recommended for thistle control are often too expensive to be used on rangeland and application of most herbicides is severely restricted or forbidden near waterways because of water quality considerations. Consequently, effective control in cropland areas is usually only temporary due to reinfestation from surrounding rangeland, roadways or waterways. In addition, farmers have recently been

forced to examine the economic tradeoffs caused by higher fuel, equipment, and herbicide costs. As they shift to more sophisticated management systems involving reduced tillage, flexible cropping, and integrated pest management, Canada thistle control could become an even worse economic problem.

With the recent successes in using plant pathogens as biological agents to control weeds, it was desirable to investigate the possibility of using a plant pathogen to control Canada thistle. It was the objective of this study to conduct a survey of endemic Canada thistle diseases in Montana and study the feasibility of using any of the associated pathogens as biological control agents of Canada thistle.

LITERATURE REVIEW

Biological Control of Weeds with Plant Pathogens

Much of the early research in biological weed control was with insects, with several well documented successes (15,120). In reference to thistles, Trumble and Kok (104) have recently reported on the exotic insects that have been introduced into North America to control *Carduus* and *Cirsium*. Interest in using plant pathogens to control weeds dates back to the late 1800's (121). In the last twenty years, this interest has increased dramatically due to economic incentives and improved understanding of the organisms involved. Today, diseases are being actively researched by several scientists (83).

Biological control can involve the use of an exotic or a native pathogen to control a weed. The classical approach involves the importation of an exotic pathogen to control an introduced or native weed. Often a weed introduced into a country is brought in without its associated diseases that help to limit the weed's population in its native habitat. This classical type of control has been successful for rush skeletonweed in Australia and pamakani weed in Hawaii (37). In the

United States, use of an exotic pathogen is reviewed by the U.S. Department of Agriculture Working Group on Biological Control of Weeds and U.S. Department of the Interior Weed Committee, and tested in quarantine facilities before being released in the United States (126).

A second tactic is the microbial or bio-herbicide approach that can apply to either a native or an approved exotic pathogen. It involves mass production and application of the pathogen to the target weed. This overcomes the natural constraints that limit the pathogen's dissemination, survival, and inoculum increase in nature. Approval of the Environmental Protection Agency (EPA) must be obtained before a pathogen can be marketed as a bio-herbicide in the United States. Within the past two years, the first two microbial herbicides, both native pathogens, have been registered by the EPA and commercialized in the United States. Phytophthora palmivora (Butler) Butler (DeVine^{TM}) is registered to control strangler vine in Florida citrus groves, and Colletotrichum gloeosporioides (Penz.) Sacc. f.sp. aeschynomene (Collego^{TM}) to control northern jointvetch in rice and soybean fields in Arkansas (56,102).

Canada Thistle

Cirsium arvense (L.) Scop. has several common names, including Canada thistle, creeping thistle, Californian thistle, and perennial thistle. This plant is a serious introduced weed of pastures, rangeland, and croplands in the northern United States and Canada. Once indigenous to southeastern Europe and the eastern Mediterranean area, it has spread throughout Europe, North Africa, and Asia, and is found in South Africa, New Zealand, and parts of Australia (67). It is now a serious weed in five countries and a principal weed in over 17 countries (48).

Canada thistle reduces crop or forage yields by competing with the crop or rangeland plants for space, light, moisture, and nutrients. It can also harbour crop insects and diseases. Canada thistle is a highly competitive weed and one of the major weeds for small grains in Montana (55,77). A thistle infestation of 2.4 shoots/m^2 may reduce the yield of spring wheat or barley by 15%, and 30 shoots/m^2 can reduce the yields of spring wheat by 60% and barley by 40% (47,77).

Cirsium arvense is in the subtribe Cardueae in the family Compositae. This perennial plant has extensive horizontal roots that overwinter and give rise to numerous aerial shoots. The shoots first form a rosette

upon emergence. Seven to eight weeks later at maturity, these shoots will have attained a height of 0.3 to 1 meter. The leaf margins can vary from deeply ruffled and lobed with numerous spines to entire margins without spines. The plants are dioecious, although the male plants can sometimes produce a few viable seeds (46). Numerous small purple flower heads form at the ends of branches. These flowers are cross-pollinated by insects, and one female plant may produce up to 1530 seeds (42).

Canada thistle seed is dispersed by wind, water, and animals, and is frequently introduced into an area by hay, contaminated crop seed or pasture seed (12,43). Recently disturbed areas such as bare land, croplands, and poorly managed pastures that are overgrazed or underfertilized are the most susceptible to colonization by seeds (13).

Once introduced into an area, C. arvense also spreads by vegetative propagation of its root system. The invasive capability of this weed is demonstrated in the ability of the root system of a single thistle to spread outwards 3 to 4 m in one season (47). This annual rate of vegetative spread is dependent on seasonal conditions, vigor of associated vegetation, soil type, and land use. In pasture lands, the annual rate of vegetative spread may vary from 4 cm to 3.4 m (13). Moreover, cultivation enhances the spread of Canada

thistle in croplands as root fragments as small as one cm long and one mm in diameter are able to produce shoots (41). This regenerative capacity of the root system, that may extend to a soil depth of over a meter, makes extermination of the weed very difficult. The best current control practices involve an integrated approach of cultural and chemical methods.

There is considerable genetic diversity in morphological and physiological characters within the species C. arvense (46,67). Based on leaf morphology alone, Wimmer and Grabowski in 1829 (122) separated C. arvense into four varieties: vestitum, integrifolium, mite (arvense), and horridum. These varieties have the same chromosome number (2n = 34) both in Europe and in Canada, and hybridize freely, so that true varietal forms are rarely found (33,38,67). Within these varieties, many clones or ecotypes have been reported that exhibit differences in photoperiodism, growth habit, vigor, seed dormancy and germination, herbicide reponse, and disease susceptiblity (38,46,67,109).

Canada Thistle Diseases

There are over forty genera of microorganisms, fungi, bacteria, nematodes, and viruses found to occur on Canada thistle in the United States and Canada, according to host indexes and a list compiled by Libscomb in 1974

(29,61,94,112). Many of these microorganisms cause only minor damage to the weed.

Puccinia punctiformis (Str.) Rohl. (= P. obtegens [Link.] Tul. = P. suaveolens [Pers.] Rost.) is the best documented of these pathogens. Systemically infected thistles are pale green, have long internodes, small leaves with incurled leaf margins, and usually produce no flowers or viable seeds (18). Interest in using this fungus as a biocontrol agent dates back to 1900 (16,28). Watson (115) and Turner et al. (108,109) have recently investigated the potential of P. punctiformis as a biological control agent for Canada thistle.

A root rot caused by Fusarium roseum (Link.) Snyder and Hans. was studied in Wyoming (62,63,86). Under natural conditions, the fungus caused wilting, yellowing, and reduced vigor in thistles. The pathogen infected a prickly denticulate-leaved thistle ecotype, but not a smooth-leaved spinulose ecotype in greenhouse studies (62).

Verticillium albo-atrum Rke. and Berth. caused a wilt of Canada thistle in Oregon and Germany (49,95). The fungus infects the roots, as well as the stem.

Detmers (33) observed bacteria that caused stunting and death of Canada thistle in Ohio, but gave no further information. Mycoplasmalike organisms have been found in Denmark and South Dakota in C. arvense plants exhibiting

chlorotic and wrinkled leaves, dwarfism, shoot proliferation, and abnormal flowers (19,89).

The disease caused by Septoria cirsii Niessl. has been reported by Detmers (33), and results in large necrotic spots on the lower leaves in mid-summer. The infected leaves turn brown and dry, and the disease slowly spreads to the upper leaves.

Another fungus, Sclerotinia, was reported to cause a wilt and crown rot of Canada thistle resulting in the death of the plant shoot (20,21,127). More information on this disease is presented in tne following section. In addition to Canada thistle, other weeds including wild mustard (Brassica kaber [Dc.] Wheeler), field pennycress (Thlaspi arvense L.), redroot pigweed (Amaranthus retroflexus L.), lambsquarters (Chenopodium album L.), sowthistle (Sonchus spp.), diffuse knapweed (Centaurea diffusa Lam.), and toothed spurge (Euphorbia dentata Michx.) have been reported to be killed by Sclerotinia spp. (20,23,70,114,127).

Sclerotinia

Taxonomy

Kohn in 1979 (58) limited the fungal genus Sclerotinia to three species, S. sclerotiorum (Lib.) de Bary, S. trifoliorum Erikss. and S. minor Jagger. The

other species previously placed in this genus were assigned to other genera.

Prior to 1980, controversy had prevailed for two decades concerning the delineation of these three species. Since 1955 when Purdy first suggested that the three species be combined into a single species, extensive studies have indicated three distinct species (81,82,117). Morphologically, special distinguishing characteristics are the small sized sclerotia of S. minor and the ascospore dimorphism of S. trifoliorum (58,110). Dissimilarities have been proven in ontogenetic, electrophoretic, mycelial interaction and cytological research (32,117,118,123,124,125). In cytological studies, Wong and Willetts (125) found for S. minor, S. trifoliorum and S. sclerotiorum, the number of nuclei per ascospore and the haploid chromosome count to be 4,4; 4,8; and 2,8, respectively. In 1983, Uhm and Fujii (111) observed 9 bivalents during S. trifoliorum ascosporogenesis. Additionally, Petersen et al. (78) have recently reported that the major sclerotia proteins vary in the three species. Serologically, the three species have common antigens (92). Differences in host range and epidemiology are discussed in subsequent parts of this section. In reviewing the host range, epidemiology and other aspects of these species, it should be remembered that identification of

S. trifoliorum in past literature may be incorrect as it was often based only on the host of origin.

Host Range

Sclerotinia occurs throughout the world, including Europe, Africa, Asia, South Pacific and North and South America, and is most common in the temperate regions. These climatic areas also enclose the geographical range of Canada thistle. Although the genus has a wide host range, corn, wheat, barley, oats, and other cereal grain crops are not affected by any of the three species (65,82).

The only host range research done to compare the three pathogens was limited to testing a small number of isolates in greenhouse studies. Keay (57), and Held and Haenseler (44) both found *S. trifoliorum* to be restricted to legumes, tomato, lettuce and beans under optimal greenhouse conditions. *Sclerotinia minor* and *S. sclerotiorum* in these tests had broad host ranges including legumes.

General field observations show *S. sclerotiorum* is most damaging to vegetables and oilseed crops, *S. minor* to peanuts and lettuce, and *S. trifoliorum* to forage legumes (98). Some field studies have shown alfalfa and red clover are not susceptible to isolates of *S. sclerotiorum* (25). In addition, Burke et al. (23)

found little damage from S. sclerotiorum in bean fields in which alfalfa had been grown for three or more years between bean crops. One explanation for these conflicting reports on host susceptibility is the diversity found among isolates within each of these species, S. sclerotiorum, S. minor and S. trifoliorum. Considerable variability in virulence is exhibited by these isolates when tested on different hosts (25,57,71,80).

Incompatible mycelial interactions have been observed between some S. sclerotiorum isolates as well as between S. trifoliorum isolates (79,91). Price and Colhoun (79,80) suggest that the differences in virulence reflect small genetic differences in the isolates.

Genetics and Pathogenesis

Sclerotinia sclerotiorum and S. trifoliorum were considered to be homothallic. Recent studies by Uhm and Fujii (110,111) have demonstrated that S. trifoliorum is partly heterothallic. This species produces dimorphic ascospores. The small ascospore strains are heterothallic and self-sterile, while the large ascospore strains are self-fertile. They also established that the microcondia produced by this fungus are functional spermatia through mating crosses between sclerotia from

the small ascospore strains and microcondia from the large ascospore strains.

A second means of genetic exchange in fungi is the transfer of genetic material between the mycelium of two strains through heterokaryosis. While the extent to which this occurs between strains of a Sclerotinia species is unknown, incompatible mycelial interactions have been observed among S. sclerotiorum isolates as well as among S. trifoliorum isolates (79,91).

The genome can also be changed through mutation. As Sclerotinia is haploid for most of its life cycle, any mutation in the genes in the genome that are expressed phenotypically would be immediately revealed.

There are many factors involved in Sclerotinia pathogenesis, the ability of Sclerotinia spp. to successfully infect a host plant and cause disease. The first step is the formation of an infection cushion leading to mechanical penetration of the host cuticle. Ascospores and S. sclerotiorum sclerotia usually require an external nutrient source to form the infection cushion. Once inside the host, the fungus produces three pectolytic enzymes and oxalic acid that work in combination to break the pectin layer between the host cells. Oxalic acid chelates cation inhibitors of pectinase activity and lowers the pH making conditions more favorable for enzymatic action. In addition,

degradative enzymes, including cellulases, hemicellulases, proteases and phosphatidase are produced by the fungus to supply nutrients for the rapid growth and development of infection hyphae (65). The differences in virulence observed between Sclerotinia spp. isolates may be a result of variations in these pathogenesis factors.

Host Resistance

Within the past 15 years, resistance to Sclerotinia has been reported in some crops. Field tolerance to S. trifoliorum has been shown in cultivars and experimental lines of alfalfa, and in cultivars of white clover (11,116). Similarly, field tolerance to S. sclerotiorum has been observed in cultivars and breeding lines of dry beans (Phaseolus vulgaris L.), and in cultivars of soybeans (14,31,39,106). Some types of tolerance are due to plant canopy structure that influences the microclimatal conditions and the development of the disease. Another type of resistance is found in the highly resistant runner bean Phaseolus coccineus L. Abawi et al. (4) transferred this resistance into P. vulgaris through backcrossing and suggested a single completely dominant gene was involved. Recently, Hunter et al. (53) screened over 400 lines of P. vulgaris for resistance using a limited-term

inoculation method under growth chamber conditions. They detected levels of resistance in lines from Central and South America, Turkey and Europe.

Resistance has been found in closely related wild plant species. Three wild Lactuca species were found to be highly resistant to S. minor (5). One of these wild species can be crossed with lettuce. Resistance to S. sclerotiorum also exists in the North America native perennial Helianthus spp., as well as in Jerusalem artichoke (H. tuberosus L.). Hybrid crosses of Jerusalem artichoke and the annual sunflower (H. annuus L.) were completely resistant in greenhouse studies (76).

Four mechanisms of host resistance to Sclerotinia have been theorized by Lumsden (65). Little research has been done in this area. Physical barriers may slow the penetration and development of the fungus, and nutritional factors within the host may be inadequate for rapid infection hyphae formation. The host may also have performed inhibitors of Sclerotinia or the host may be induced by the fungus to form phytoalexins.

Infection

Sclerotinia spp. can survive and infect over wide temperature and pH ranges. Depending on the species involved, disease can result from airborne ascospore

infection or soilborne mycelial infection. No asexual spores are produced.

Ascospores are the prime source of infection in diseases caused by both S. sclerotiorum and S. trifoliorum, but of limited importance in the epidemiology of S. minor caused diseases. In the latter case, soilborne mycelial infection is important (2).

Ascospores are released from apothecia produced by sclerotia. Much of the following information on ascospore infection is based on S. sclerotiorum. Less is known about S. trifoliorum ascospore infection, that usually occurs in the autumn. Apothecia initiation is closely tied to soil depth and soil moisture. Only preconditioned sclerotia within the top 3 to 5 cm of the soil surface are capable of forming apothecia, as stipes over this length are rarely produced (2,30). Furthermore, continuous soil moisture for 10 to 14 days is required for apothecia to form. Sclerotinia sclerotiorum isolates from New York State required a soil matric potential of -80 to -160 millibars (mb), while isolates from Nebraska required -250 mb for optimal apothecia production (2,98). In California and other southern areas, S. sclerotiorum apothecia are produced in late winter through early spring. At more northerly locations, ascospores are formed throughout the growing season provided the top 3 cm of the soil is near

saturation. In the northern semi-arid regions, apothecia formation does not occur until early summer when the plant canopy has covered the soil surface decreasing water evaporation (2,72,98).

Sclerotinia sclerotiorum ascospores require 48-72 hours of continuous free water and an exogenous food source such as pollen, dying flower petals, or senescing or injured plant tissue to infect a host (1,30). The lack of this exogenous energy source prevents infection. Ascospores have been found to survive 12 days in the field in New York state (2,24). In vitro studies have shown survival is enhanced by temperatures below 20° C and relative humidities below 35% (24). Free water is also required for lesion expansion. If the surface of infected tissues, except for bulky stem tissue, becomes dry, lesion enlargement is abruptly stopped until free water is again available (2).

Soilborne mycelial infection results from mycelial germination of sclerotia. This is the principal means of infection in S. minor and no outside food source is required (2). While the optimal soil water potential for mycelial germination of S. minor sclerotia is the same as for carpogenic (apothecial) germination of S. sclerotiorum sclerotia at -250 mb, germination can occur as low as -2 bars (3). In addition, prior drying of S. minor sclerotia at the soil surface, stimulates

mycelial germination. With S. sclerotiorum, the importance of mycelial sclerotia germination depends upon the crop involved. In beans, this type of infection is of minor importance and occurs only when an exogenous energy source is in direct contact between the sclerotium and the host tissue (1). It is a different case in sunflowers, where the basal stem rot phase of Sclerotinia wilt is due entirely to mycelial infection from germinating sclerotia (51,52). With sunflowers, no exogenous food source is needed. Recently, host root exudates have been implicated in influencing the type of sclerotia germination. Rimmer and Menzies (87) found rape and corn seedling exudates could affect mycelial and carpogenic germination of single spore isolates of S. sclerotiorum. Sclerotia placed with sunflower plants had increased mycelial but reduced carpogenic germination, in comparison to sclerotia placed with rape plants.

Sclerotinia sclerotiorum and S. minor have been described as simple interest pathogens as defined by Van der Plank (2,113). Nonetheless, some secondary spread may occur from contact with mycelium from infected plants, including spread through root contact or interconnecting roots. With S. sclerotiorum, actively growing mycelium on moist infected plant tissue requires only 16 - 24 hours of leaf wetness to infect a new host

plant, whereas dry infected plant tissue requires over 72 hours of continuous moisture (1).

Dissemination

In the Sclerotinia diseases where ascospore infection is important, windblown ascospores may spread the fungus from field to field. The ascospores are discharged in a mucilaginous material that cements them to any object encountered. Most of the ascospores are trapped by the plant canopy, but some will escape to above the crop canopy and be carried in air currents. Spores have been trapped at distances of 150 m to several hundred meters from the nearest source (111,119). Hims (45) observed an outbreak of *S. sclerotiorum* restricted to one corner of a 23 ha field of oilseed rape and suggested that the source of inoculum was ascospores from weeds in an adjacent woodland. Ascospores can also be transmitted with pollen by honeybees, and in irrigation runoff water (97,100). Spores of *S. sclerotiorum* have been shown to survive up to 5 days in deionized water (99).

Sclerotia dissemination is limited. In *S. minor* where the sclerotium is the infective propagule, dispersal is generally restricted to secondary plant to plant spread. Sclerotia can, however, be moved from field to field with machinery and in irrigation runoff

(90). It is possible for S. sclerotiorum sclerotia to remain viable up to 21 days in flowing ditch water but longer periods may destroy the sclerotia (69,99). Animals eating Sclerotinia infested crop refuse containing masses of sclerotia may transmit the fungus at very low levels. Brown (22) found only 2% of the sclerotia still intact and viable after passage through sheep. Sclerotinia can also be spread in seed lots contaminated with sclerotia or with mycelium infected seed. Steadman (96) found S. sclerotiorum mycelium in 12% of abnormal chalky, discolored bean seed and 0.5% of normal bean seed.

Survival

Sclerotinia is a facultative saprophyte and grows rapidly in pure culture. Yet, this fungus is not a vigorous competitor in the soil, and aside from invading necrotic or senescent plant parts, colonizes only plant tissue previously killed by the pathogen. Cook et al. (30) found S. sclerotiorum was unable to survive as mycelium on soil plant residues but could overwinter as mycelium in 2% of the bean seeds left on the soil surface after harvesting. Sclerotia are the principal means Sclerotinia survives environmental extremes.

Many studies have been done on Sclerotinia sclerotia survival in the soil. Due to non-comparable soil and

environmental conditions, secondary sclerotia formation as well as unrelated soil microflora and methodology, the results have varied considerably, with survival times from a few weeks to 8 years. Based on growers' experience with crop rotation, sclerotia of S. trifoliorum survive in nature for about 8 years, and sclerotia of S. sclerotiorum and S. minor for 4 to 5 years (6). The use of crop rotation to nonsusceptible hosts as a control, may be of limited value unless all inoculum sources and susceptible weed hosts are eliminated.

Various factors may be reducing sclerotia viability in nature. Troutman and Matejka (103) noted no build-up of S. sclerotiorum sclerotia over a 12 year period in irrigated lettuce plots in Arizona, despite being inoculated each year with the fungus. Destruction by soil microorganisms is one of these factors and appears to be greatly affecting sclerotia survival. A natural decline of S. sclerotiorum was observed in potato fields in Oregon, recently put into production with the installation of center-pivot irrigation. Some of these fields had moderate to severe sclerotinia stem rot and only 0 to 16% sclerotia infection from the hyperparasite Sporidesmium sclerotivorum Uecker, Ayers and Adams. Another field had little stem rot but over 92% sclerotia infection by S. sclerotivorum (7).

Sporidesmium sclerotivorum, as well as other microorganisms, are being studied as possible Sclerotinia biological control agents. Much of this work has been done within the past five years. In field testing, Sporidesmium sclerotivorum provided 40 to 83% disease control of sclerotinia lettuce drop caused by Sclerotinia minor over a three year period, and reduced the sclerotia populations by 75 to 95% (9). This mycoparasite grew intercellularly through the sclerotium, did not invade sclerotial cells, and produced large quantities of macroconidia on the sclerotium surface (10). Methods to cultivate this fungus have now been patented (8). Another fungus, Talaromyces flavus (Klocker) Stolk and Samson reduced the percentage of Sclerotinia infected sunflowers to 27 and 4% in field trials, verus 81 and 47% disease in the controls (66). Coniothyrium minitans Camp., Trichoderma koningii Oud. and Teratosperma oligocladum Uecker, Ayers and Adams also greatly reduce the number of viable sclerotia and show potential as biological control agents of Sclerotinia spp. (17,36,50,105,107).

MATERIALS AND METHODS

Disease Survey

A disease survey was conducted in Montana to find diseased Canada thistle plants in the fall of 1980 and spring and summer of 1981. Isolations were done in the laboratory using water agar, potato dextrose agar and BCBRVB media (69,88). Some of these isolates were tested on young Canada thistle plants in the greenhouse. The thistle plants had previously been started from thistle root sections placed in pots containing a sand-steamed soil mix. Three inoculation techniques were attempted to obtain successful infection of Canada thistle with the fungal isolates.

1. The thistle plant was put in a mist chamber for 24 hours, sprayed with a suspension of mycelial fragments and possibly spores, then either covered with a plastic bag or placed back in the mist chamber for 2 days.

2. Mycelial fragments were applied to the soil around the crown of the thistle plant and then covered with a 2.5 cm layer of sand.

3. A mycelial agar plug was placed next to the uninjured thistle stem and held in place with a piece of parafilm.

If the organism was a bacterium, a bacterial suspension was injected into the stem of the Canada thistle plant.

Hypersensitivity tests on tobacco were also done with the bacteria isolates (93).

A Septoria sp. was tested in the field. The fungus was grown in a potato dextrose broth culture on a shaker for 5 days. The mycelium was fragmented in a blender, diluted with distilled water and sprayed on 1.8 X 1.8 m field plots of Canada thistle during June.

Sclerotinia

In addition to the Sclerotinia isolates found during the disease survey of 1981, a search was conducted in Montana during the summer of 1982 for virulent isolates of Sclerotinia from Canada thistle. These isolates were stored on potato dextrose agar slant tubes at 5° C and also as sclerotia from dried wheat kernel cultures.

To determine the species of the Sclerotinia isolate selected for the field trials, air-dried sclerotia from wheat kernel cultures were surface sterilized and plated on 1% tap water agar. The plates were sealed with parafilm and incubated in the dark at 5° C for over four weeks until stipes formed. The plates were then incubated for three weeks at 20° C and illuminated by two 20W cool white fluorescent lamps, to obtain apothecia formation.

Inoculum Preparation

An inoculum of *Sclerotinia* infested wheat or oat kernels was utilized in all of the greenhouse and field studies. The oat kernel inoculum was used in all the greenhouse experiments and in the Fall 1981 Field Trials. Quart mason jars (with filter paper lids secured with screw rings) containing 125 grams of oat kernels and 175 ml of distilled water were autoclaved at 121° C and 1.4 kg/cm^2 pressure for one hour, inoculated with one 7 mm diameter mycelial plug of the desired isolate, and incubated at room temperature for two weeks. Sclerotia formed in the cultures. The inoculum was applied fresh in the Fall 1981 Field Trials. For the greenhouse experiments, the inoculum was air-dried for 5 days and stored at room temperature. A wheat kernel inoculum was used for the 1982 Field Trials. This inoculum was prepared similarly, except 250 grams of wheat kernels and 325 ml of distilled water were used per quart jar. Also, the inoculum was air-dried and stored at room temperature until needed. The percentage of viable inoculum was not determined. Sterile oat or wheat kernels were used for the control treatments.

Greenhouse Studies

Pathogenicity on Canada Thistle

Ten Sclerotinia isolates obtained from diseased Canada thistle, bean or safflower hosts during the 1981 Disease Survey (Table 1) were tested on Canada thistle. Isolate 81-167-04 from Canada thistle, produced small sclerotia, and was identified as S. minor. Canada thistle root pieces had been collected from one small area of a Canada thistle patch representing one ecotype of variety horridum. These roots were cut into 5 to 10 cm sections and the cut ends sealed with paraffin wax. Two root pieces were planted in each 16.5 cm pot containing a 1/3 sand and 2/3 steamed soil mix. Plants 6 to 8 weeks old and in the vegetative stage of growth were inoculated by placing one infected oat kernel against the crown of the plant at a soil depth of 0.5 to 1.0 cm. There were two plants per pot, and thirty plants were inoculated per isolate. Sterilized non-inoculated oat kernels were used on the control plants. Pot placement was not randomized but grouped according to the isolate treatment.

These tests were conducted when the greenhouse had 9 hours of natural light and the temperature ranged from a minimum of 14° C to a maximum of 20 to 26° C. Each day the number of dying and dead plants was observed, and

Table 1. *Sclerotinia* isolates collected during the Canada Thistle Disease Survey and used in pathogenicity, host range and field studies.

Isolate number	Collection site	Host	History of collection site
81-85	Bozeman	Canada thistle	Pasture, former landfill
81-154-01	Bozeman	Canada thistle	Previously cropped to faba beans, iris
81-155-01	Bozeman	Canada thistle	Pasture, former landfill
81-162-01	Huntley	Canada thistle	Multicropped, thistle research area
81-164-01b	Reedpoint	Canada thistle	Rangeland
81-167-01	Springdale	Canada thistle	Pasture
81-167-04	Springdale	Canada thistle	Pasture
81-171	Sidney	Safflower	Irrigated safflower, sugar beets, beans
81-177-04	Bozeman	Canada thistle	Vacant land
81-178	Bozeman	Bush bean	Home garden

after 43 to 48 days, each plant was scaled according to the conditions of the thistle plant. This disease scale was based on whether the root piece, and/or above ground portion of the plant had died, and on the length of the lesion at the site of inoculation.

Host Range Studies

The same ten isolates of Sclerotinia, examined in the pathogenicity study on Canada thistle, were tested on eleven types of crop plants (Table 2). Canada thistle, was not included in the host range studies. The seeds were planted in a sand-steamed soil mix in 5 cm to 16.5 cm diameter pots. The seedlings were thinned to one to eight plants per pot, depending on the crop and pot size, and inoculated at the seedling stage when 6 to 17 days old. Potatoes were grown from untreated tuber discs that were planted into 30 X 50 cm flats containing a sand-steamed soil mix. They were inoculated 18 days after emergence. Thirty or thirty-two plants of each crop were tested with each isolate or control. The plants were inoculated by placing one infected kernel into the soil at the crown of the plant. The pot placement was randomized for each crop and daily observations were taken on the number of wilting plants. The tests were ended after three to four weeks when the mortality curve had leveled off.

Table 2. The crop cultivars tested for susceptibility to *Sclerotinia sclerotiorum*.

Crop	Cultivar
Safflower	S-541
Bush bean	Bush Blue Lake
Corn	B 37(N)
Sunflower	Dalgren 704
Barley	Clark
Oats	Otana
Pinto bean	UI III
Faba bean	Ackerperly
Lentil	Chilean
Wheat	Newana
Potato	Russet Burbank

The studies were conducted in the summer when the greenhouse had 14 to 16 hours of natural light. The temperature ranged from a minimum of 13 to 16° C to a maximum of 28 to 32° C.

To test isolate stability, isolate 81-171 from safflower was run through two consecutive inoculation and reisolation cycles on Canada thistle before being tested on Canada thistle, safflower, sunflower, and bush bean. The same isolate was also transferred eleven times on potato dextrose agar in the laboratory before being tested on Canada thistle and safflower. This experiment was conducted at the same time and under identical procedures as the host range experiment except Canada thistle was also included in the test.

Fall 1981 Field Trials

The trials were located at the Arthur Post Research Center west of Bozeman. The area was well drained and good cropland of soil type Amsterdam silty clay loam (fine-silty, mixed Typic Cryoboroll). Thistles had been planted at the site in 1979, and no herbicides applied since this time. The area was cultivated in August 1981 and the thistle shoot density averaged 40 shoots/m^2 at the time of treatment.

The plot design was completely randomized. Fresh oat kernel inoculum of *S. sclerotiorum* isolate 81-162-01 was applied October 21, 1981. This inoculum was mixed into the upper 2 cm of the soil of 2 X 2 m plots at three different rates, with four replications of each rate. Sterilized non-inoculated oat kernels were applied at the same 3 rates, with four replications of each rate. The three rates of application used were 96 g/m^2, 12 g/m^2 and 1.5 g/m^2 based on the weight of the fresh infested oat kernel inoculum. This corresponds to 3125, 400 and 50 kernels/m^2, respectively. The thistles were in the rosette stage of growth at the time of inoculation. Data were collected after 21 days, on November 11, by counting the number of dead thistle shoots and the total number of shoots for each plot.

No further application of *S. sclerotiorum* was applied to the plots, and they were monitored during the

1982 growing season. Infected thistle shoots were staked as they died, since dead shoots soon decompose. Once disease symptoms appeared, data were collected on July 18 and August 22 by counting the number of dead shoots in each plot. A healthy shoot count was taken only on July 18. To compare any changes in thistle density from 1981 to 1982, the thistle densities were transformed to log (x) and analysed by a 2x2x3 factorial analysis of variance and non-factorial comparisons. To investigate differences in the percentage of dead shoots among treatments an arcsine transformation, and 2x3 factorial analysis of variance and non-factorial comparisons were used. A correlation analysis (significant, P = 0.05) was ultilized to find any correlation between % dead shoots in 1981 and % dead shoots in 1982.

1982 Field Trials

During the 1982 growing season, field studies were conducted at the Fort Ellis Station east of Bozeman. These studies consisted of two experiments: Timing of Inoculum Application and Soil Incorporation of Inoculum. Dried wheat kernel inoculum of *S. sclerotiorum* isolate 81-162-01 was used in both experiments. Two rates of inoculum application were tested, 83 g/m^2 and 8 g/m^2 based on the weight of the dried infested wheat kernel inoculum. This corresponds to 3125 and 313

kernels/m^2, respectively. Sterilized non-inoculated wheat kernels were applied in the control plots. All plots were 2 x 2 m. Over the time period of these experiments, the infected thistle shoots were staked as they died. Data were collected by counting the number of staked dead thistle shoots at one to four week intervals during the growing season. Healthy shoot counts were taken from August 17 to 21 for each plot. In some plots, healthy shoot counts were also taken on June 26, 27, and July 28, 29. The final percentage of dead shoots was calculated for each plot, based on the number of dead shoots on August 17 to 21, of the total number of shoots. Specific details of each experiment are described under subsequent subheadings.

With no further inoculum application, close surveillance was kept the following year during the 1983 growing season for the development of Sclerotinia disease symptoms. To assess the damage to the Canada thistle root system, the 1983 thistle shoot density was calculated for each plot and compared with the 1982 density values. This data was collected July 27 to 29, 1983, by counting the number of thistle shoots in a 1.2 X 1.2 m area within the center of each plot.

To test for the presence of airborne ascospores during 1983 at the site of the 1982 Field Trials, methodology developed by Williams and Stelfox (119) was

ultilized. Nine cm diameter petri plates of potato sucrose agar were taped to vertical wood stakes 15 cm to 1 m above the soil surface on July 19, 20, 22, 23, 24 and 27 at 7:00 am. Five hours later the plates were collected and incubated at room temperature.

The experimental site was in an isolated low lying but drained area, naturally infested with Canada thistle. Cereal crops had been previously grown on this land, and the site was cultivated in the fall of 1981. The density of Canada thistle shoots varied over the site. In the experimental plots, the density ranged from 24 to 85 shoots/m^2, and according to the variety descriptions by Moore and Frankton (68), contained ecotypes belonging to the three varieties, horridum, arvense and vestitum.

Timing of Inoculum Application

This experiment was designed to determine the optimal time to apply the S. sclerotiorum inoculum. The experimental plan was a randomized complete block with four replications of each treatment. There were three application timing treatments, May 24, June 18 or July 4, 1982. At these times, the thistles were respectively in the rosette stage, vegetative stage, and flower bud stage. In addition, two rates of dried inoculum application were used, 3125 and 313 kernels/m^2. Sterilized non-inoculated wheat kernels were applied at

the same rates and times in the control plots. The inoculum was hand-broadcast over the surface area of the plot. Only one application of the inoculum or control kernels was made in any one plot. There were 12 treatments, replicated four times for a total of 48 plots.

To detect any statistical differences in the percentage of dead shoots among the treatments, an arcsine transformation followed by a 2x2x3 factorial analysis of variance and non-factorial comparisons were used. The data was further analysed by a 2x3 factorial analysis of variance that excluded the control treatments, and linear and quadratic comparisons. Differences in thistle density were identified by logarithmically transforming the data and doing a 2x2x2x3 factorial analysis of variance, non-factorial comparisons and separate 2x2x3 factorial analysis of variances of each level of the treatment factor.

Soil Incorporation of Inoculum

To investigate the effect of incorporating the *S. sclerotiorum* inoculum into the soil, soil incorporation was compared to surface application. The test area had been cultivated May 17, 1982, in addition to the fall 1981 cultivation, and the dried inoculum applied May 23, 1982 before the thistle shoots had

emerged. The experiment was completely randomized with four replications of four treatments. The inoculum was hand-broadcast over the soil surface or mixed with a hand rake into the top 3 cm of the soil. The 3125 kernels/m^2 rate of application was used. The remaining two treatments were controls, where sterilized non-inoculated kernels were similarly applied to the soil surface or incorporated into the soil.

An arcsine transformation and 2x2 factorial analysis of variance were ultilized on the % dead shoots data to discover any differences among the treatments. The thistle density data was transformed to log (x) and analyzed by a 2x2x2 factorial analysis of variance and non-factorial comparisons.

Mycelial Spread

A Canada thistle shoot in the center of a 2.6 X 2.6 m plot was inoculated at the plant crown with five *S. sclerotiorum* infected kernels. Four plots were inoculated May 25, 1982 and four more at the end of June 1982. Observations were taken on the mycelial spread of the fungus, by noting the distance of *Sclerotinia* infected thistles from the initial inoculated thistle.

Statewide Research Trials

Research field trials were set out at eight different locations in Montana in 1982, to test

S. sclerotiorum under varied environmental and habitat conditions. These locations included Havre, Moccasin, Benchland, Conrad, Creston, Corvallis, Huntley, and Circle (Table 3). At each location, the 2 X 2 m plots were completely randomized. Four plots were inoculated with dried wheat inoculum of S. sclerotiorum isolate 81-162-01 at the 3125 kernels/m^2 rate. Four other plots were inoculated with sterilized non-inoculated wheat kernels. The sites were inoculated in 1982 on the following dates: Circle May 31, Havre June 2, Corvallis June 7, Moccasin June 14, Benchland June 16, Conrad and Creston June 21, and Huntley July 2.

Data were collected at five of the sites by counting the number of dead thistle shoots in each plot. This count was then compared to the total number of shoots in each plot. The difference in percentage of dead shoots between the S. sclerotiorum treated and control plots was tested by the Student's t test. Prior to the statistical test, an arcsine transformation was used and the variances of the two treatments were tested for homogeneity. The other three sites, at Conrad, Creston and Circle, were only checked once and no data were collected.

Thistle density data was also collected at two of these sites, Corvallis and Benchland, on July 1 and July 14 of 1983, respectively. A logarithmic (x+1)

Table 3. Site histories of the Statewide Research Trials to test for ability of *Sclerotinia sclerotiorum* to control Canada thistle.

Location	Site[a]	Land Use	Thistle density[b] (shoots/m^2)
Havre	Northern ARC	Pasture (not grazed)	7 to 16
Moccasin	Central ARC	Dryland barley	35 to 73
Benchland	2 mi from town	Pasture (grazed)	59 to 162
Corvallis	Western ARC	Fruit orchard (cultivated 1982)	10 to 19
Huntley	Southern ARC	Cropland, thistle research area	15 to 60
Conrad	Western Triangle ARC	Dryland barley	*
Creston	Northwestern ARC	Pasture (grazed)	*
Circle	R. Jensen Farm	Pasture (grazed)	*

[a]ARC = Agricultural Research Center.
[b]No data were obtained from the starred locations.

transformation was done on this data, followed by a 2x2 factorial analysis of variance and non-factorial comparisons.

Four additional plots were set out at each of the eight research field trial locations to measure the spread of the fungus. Five infested kernels were placed 1 cm into the soil at the center of a 2.6 X 2.6 m plot next to a Canada thistle shoot. These plots were inoculated in 1982 on the same dates as the previous experiment mentioned for each trial location. The distance of Sclerotinia infected thistles from the initial inoculated thistle was noted.

Another field trial was set out at the Huntley site on May 19, 1983. The land, naturally infested with Canada thistle, had not been cultivated since June of 1982. The treatments consisted of two application rates of fresh wheat inoculum of S. sclerotiorum isolate 81-162-01, 260 and 26 g/m^2, and a control treatment. The two rates correspond to 3125 and 313 kernels/m^2, respectively. Each treatment was replicated four times and the 1.5 x 2.6 m plots completely randomized. The number of dead shoots was observed on June 20 and August 22, 1983. The difference in percentage of dead shoots between the treatments was tested for both the June 20 and August 22 data by a one-way analysis of variance and Newman-Keul's multiple range test. An arcsine

transformation was performed on the data prior to statistical analysis.

RESULTS

Disease Survey

Six pathogens were found to occur on Canada thistle in Montana. These included *Puccinia punctiformis*, and pathogens belonging to the following genera: *Septoria*, *Fusarium*, *Sclerotinia*, *Pseudomonas*, and *Alternaria*.

A *Septoria* sp. was found at several locations. Canada thistle plants severely infected with *Septoria* were smaller in size, had black stem lesions, and leaves that were curled with large necrotic lesions. This fungus was never found to infect the underground portion of the plant. Pycnidia were produced on potato dextrose agar, water agar, or autoclaved wheat kernels if these cultures were incubated at 15° C for 8 weeks. Pycnidia were dark brown, globose, ostiolate and 4.2 to 4.8 mm in diameter. The hyaline conidia were elongate to filiform in shape and slightly wider at the top (25 µ) than at the base (10 - 15 µ). These spores were 520 to 720 µ long, and 7-celled. Disease symptoms appeared in the new growth of young Canada thistle plants in the greenhouse that were sprayed with a fragmented mycelial suspension of the fungus. The fungus moved systemically in the plant causing stem lesions and the leaves to curl, with

the inside margin of the curled leaf becoming necrotic. The plant was able to outgrow the disease under greenhouse conditions. Septoria was re-isolated from these inoculated plants. Disease symptoms appeared in field plots sprayed with a mycelial suspension of Septoria, but the disease did not stop seed production or thistle growth.

Fusarium was isolated from diseased Canada thistle roots but produced no symptoms on Canada thistle plants inoculated with the fungus in the greenhouse.

Pseudomonas was also isolated from diseased Canada thistle plants. Some fluorescent isolates gave a hypersensitive reaction when tested on tobacco leaves, especially those isolated from Puccinia punctiformis infected thistles. No symptoms were observed when these isolates were stem inoculated into young Canada thistle plants in the greenhouse.

An Alternaria sp. was found to occur on the lower senescing leaves of Canada thistle plants. This Alternaria did not reduce thistle growth based on field observations.

Sclerotinia was researched in more detail as described in the following studies.

Sclerotinia

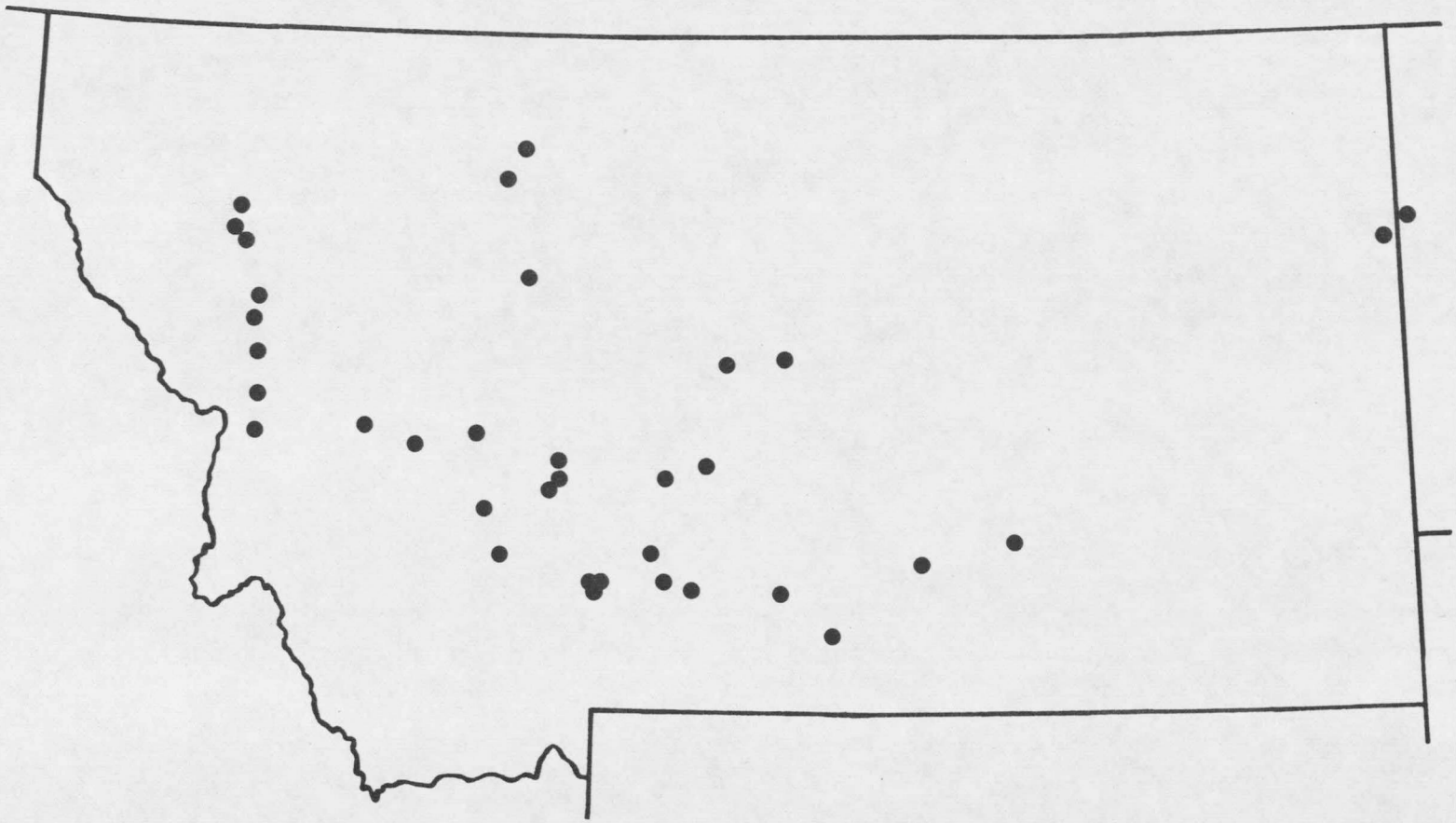
Natural Occurrence

Under natural field conditions, Sclerotinia apparently causes small patches of dead Canada thistle plants ranging from one dead plant up to an area of 1.5 X 1.2 m. Disease symptoms of wilt and subsequent death of the above ground portion of the thistle plant were first observed in late June to July. At one undisturbed collection site located at Bozeman, Montana, the 1.5 X 2.4 m center of a Sclerotinia infected Canada thistle patch was almost free of thistle shoots. Surrounding this center was a ring of thistles dying from Sclerotinia infection.

The fungus was isolated from the crown area and upper portion of the root system. Sclerotinia was found to extend as far as 35 cm into the root system, sometimes including the horizontal root. The infected root tissue had a shredded appearance and sclerotia formed on both the inside and outside of the upper root. Rarely was the fungus isolated from above the crown of the thistle plant.

Sclerotinia isolates were collected from thirty-five different locations in Montana during the 1981 and 1982 Disease Surveys (Figure 1). These included three S. minor isolates isolated from Canada thistle, that

Figure 1. Location of Sclerotinia samples collected from 35 sites in Montana and North Dakota during the 1982 Canada Thistle Disease Survey.



produced small-type sclerotia. While it has not been determined whether most of the remaining isolates were *S. sclerotiorum* or *S. trifoliorum*, the isolate (81-162-01) used in all of the field trials was *S. sclerotiorum*. This isolate displayed no dimorphism in ascospore size upon investigation of laboratory produced apothecia. *Sclerotinia* diseased Canada thistles were found in pastures, rangelands, waste areas, and along the edges of alfalfa, wheat, barley, and safflower fields. The fungus was also isolated from two other weeds, bull thistle (*Cirsium vulgare* [Savi.] Tenore) and prickly lettuce (*Lactuca scariola* L.).

Pathogenicity Studies on Canada Thistle

In greenhouse studies, the oat or wheat kernel inoculum had to be placed against the crown or root of the thistle plant to get infection. No prior wounding of the plant tissue was required.

There was considerable variation in virulence between the ten isolates when tested on Canada thistle, especially between the isolates from Canada thistle hosts and the isolates from safflower or bean hosts. Wilting symptoms appeared within 3 days after inoculation. After 20 days the number of dying thistles had stabilized. Six isolates from Canada thistle killed from 77 to 97% of the inoculated thistle shoots, and about 60% of the roots

(Figure 2, Table 4). In comparison, the isolate from safflower killed 37% of the shoots while the isolate from bean killed only 13%. Isolate 81-154-01, from thistle in an area previously cropped to faba beans and iris, and S. minor isolate 81-167-04, were much less virulent than the other isolates from Canada thistle.

Sclerotinia lesions developed at the crown of some of the inoculated Canada thistle plants that did not have wilting symptoms. One inoculated thistle plant started to wilt but recovered. Upon close examination of this plant, a root had developed above the infected upper root zone.

Host Range Studies

Potato plants and corn, barley, oat, and wheat seedlings showed no disease symptoms when tested with the ten Sclerotinia isolates in greenhouse studies. Canada thistle, sunflower, and safflower were the most susceptible to Sclerotinia. Lentils and bush, pinto, and faba beans were affected to a lesser degree. The potatoes showed symptoms of herbicide damage which is suspected to be due to use of contaminated soil or to a tuber source. Differences in virulence were found between the isolates on the hosts tested (Table 4). The isolate from bean was not highly virulent and inoculum of isolate 81-155-01 was not viable.

Figure 2. Pathogenicity of Sclerotinia isolates on Canada thistle in greenhouse studies.

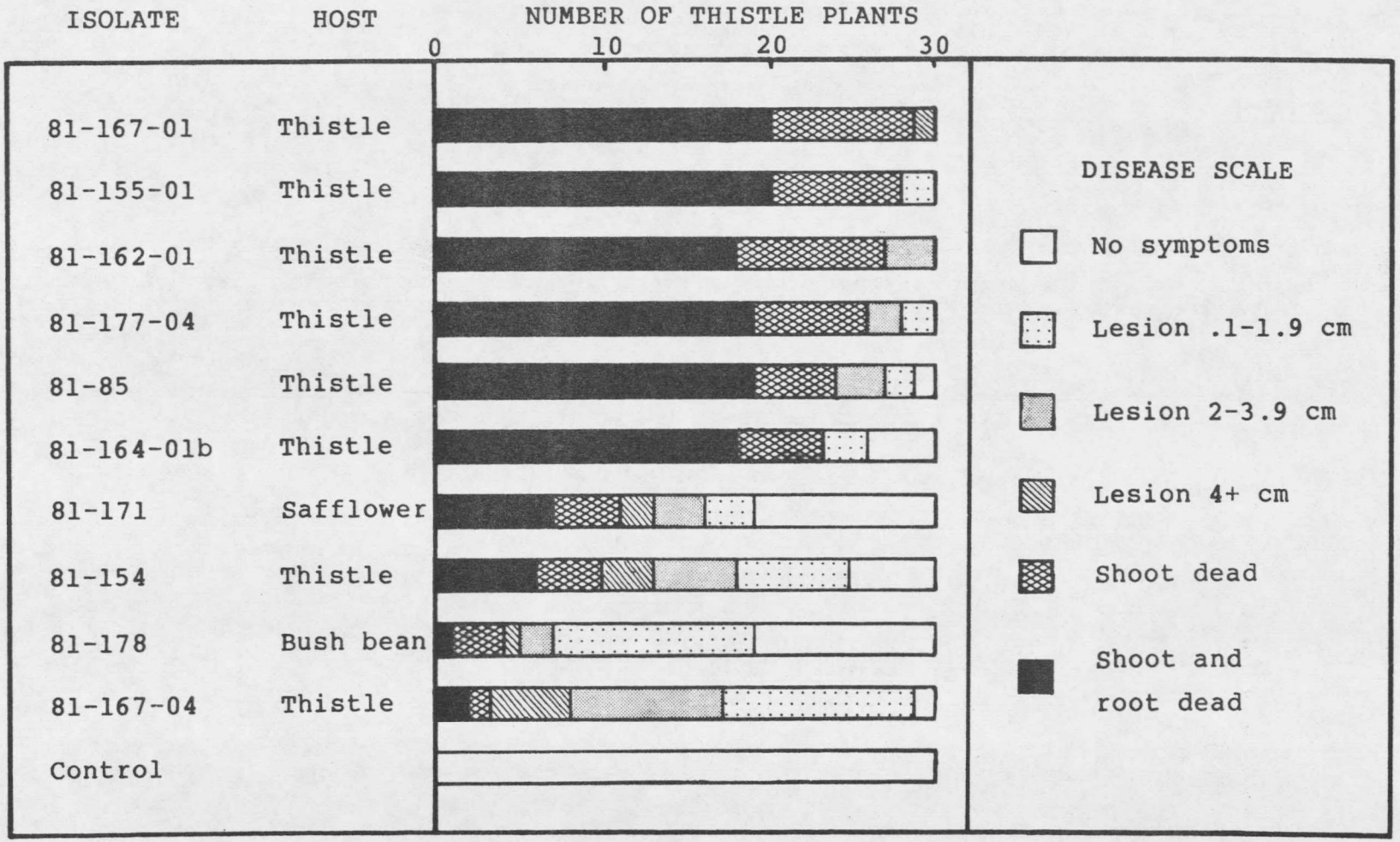


Table 4. Pathogenicity of *Sclerotinia* isolates to Canada thistle, and various other hosts.

Isolate Number	Host	% dead plants[a]							
		Canada thistle[b]	Sunflower	Safflower	Lentil+	Bush bean	Pinto bean+	Faba bean+	Potato+corn,oats+ barley+,wheat
81-167-01	Canada thistle	97	63	53	31	23	19	3	0
81-155-01[c]	Canada thistle	93	*	*	*	*	*	*	*
81-162-01	Canada thistle	90	80	63	47	23	13	3	0
81-177-04	Canada thistle	87	73	47	16	13	22	0	0
81-85	Canada thistle	80	63	23	31	3	19	0	0
81-164-01b	Canada thistle	77	77	40	3	10	9	0	0
81-171	Safflower	37	73	50	25	17	6	0	0
81-154-01	Canada thistle	33	47	40	25	7	3	0	0
81-178	Bush bean	13	3	0	0	0	0	0	0
81-167-04[d]	Canada thistle	10	7	10	0	0	0	0	0
Control		0	0	0	0	0	0	0	0

[a]Thirty plants of each crop type were tested for each isolate, except the crops marked by "+", where thirty-two plants were tested for each isolate.
[b]The Pathogenicity Study on Canada thistle was a separate experiment from the Host Range Studies.
[c]The inoculum used on the starred crop types was not viable.
[d]This isolate produced very small sclerotia and was identified as *S. minor*.

Repeated cycling of isolate (81-171) from safflower on a Canada thistle host, later proved to increase the virulence on three of the four hosts tested: Canada thistle, sunflower, and safflower, but not on bush bean (Table 5). Transferring the same isolate eleven times in culture prior to the experiment decreased its virulence on both of the plants tested: Canada thistle and safflower.

Table 5. Virulence of *Sclerotinia* isolate 81-171 from safflower after repeated cycling on Canada thistle or transferring in culture.

Treatment	% dead plants			
	Canada thistle	Sunflower	Safflower	Bush bean
Original isolate	60	73	50	17
Cycled on Canada thistle[a]	83	87	77	13
Transferred in culture[b]	43	*[c]	23	*
Control	0	0	0	0

[a]Isolate 81-171 was cycled twice on Canada thistle plants in the greenhouse prior to the experiment.
[b]Isolate 81-171 was transferred eleven times in culture on potato dextrose agar prior to the experiment.
[c]Not tested.

Fall 1981 Field Trials

This study investigated the ability of S. sclerotiorum to infect Canada thistle under October environmental conditions. The thistles were in the rosette stage of growth. During this time the air temperature ranged from a maximum of 2 to 19° C, and a minimum of -9 to 9° C, and the soil temperature at a depth of 5 cm ranged from a maximum of 4 to 8° C and a minimum of 1 to 6° C (27). Under these conditions, the thistle plants were subjected to frost injury. Wilting symptoms appeared within 10 days of inoculum application.

After three weeks the percentage of dead thistle shoots ranged from 35 to 60% in the plots inoculated at the 3125 kernels/m^2 rate, which was significantly different from the control plots that averaged 1% (Table 6,7). The lower application rates did not give high rates of shoot kill and the 50 kernels/m^2 rate was not significantly different from the control plots. The percentage of dead thistle shoots was low in all of the control plots and not affected by increasing amounts of non-infested sterilized kernels. The fungus was found to occur in the crown of infected plants but did not extend into the root system. Fifteen days after these readings were taken, the soil froze down to the 5 cm depth and the ground remained frozen until spring. The coldest air

Table 6. The effect of an October *Sclerotinia sclerotiorum* treatment on Canada thistle mortality and thistle density[a].

Rate[b] k/m^2	% dead shoots November 1981		Change in thistle density (1982 density ÷ 1981 density)[c]		% dead shoots August 1982	
	Treated	Control	Treated	Control	Treated	Control
3125	45 ± 1 (35-60)	.5 ± .6 (0-1)	1.20 (.93-1.80)	1.20 (.67-1.82)	38 ± 13 (26-56)	5 ± 5 (1-11)
400	9 ± 9 (0-20)	.3 ± .5 (0-1)	1.58 (.89-2.61)	1.33 (1.17-1.42)	22 ± 17 (8-45)	5 ± 4 (1-11)
50	2 ± 2 (0-5)	.5 ± 1 (0-2)	1.22 (.94-1.95)	1.16 (.93-1.36)	4 ± 2 (2-7)	4 ± 2 (2-6)

[a]Mean ± standard deviation, with range in parenthesis.
[b]Fresh inoculum of isolate 81-162-01 was applied October 21, 1981. k = kernels
[c]*Sclerotinia* infected thistles were included in the thistle densities. Thistle densities ranged from 13 to 57 shoots/m^2 in 1981.

Table 7. Statistical analysis of % dead thistle shoots[a] in November 1981 and August 1982 after *Sclerotinia sclerotiorum* treatment in October 1981.

Source of variation	df	Mean square[b] November 1981	August 1982
2x3 factorial ANOV:			
Treatment[c]	1	2142.8**	1139. **
Rate	2	739.9**	361.1**
Treatment x rate	2	653.1**	361.5**
Non-factorial partition:			
Among rates treated	2	1390.8**	722.5**
Among controls	2	2.1	.2
Treated versus controls	1	2142.8**	1139. **
50 k/m^2 rate treated vs control	1	43.5	.06
Error	18	37.9	50.2

[a]Arcsine transformation of % dead shoots. df = degrees of freedom, k = kernels.
[b]** = significant, P = 0.01.
[c]Treatment = control or *S. sclerotiorum* treatment.

temperature recorded during the 1981-1982 winter was -31° C.

In 1982, thistle shoot density increased significantly over the 1981 densities in both the S. sclerotiorum and control treatments (Table 6,8). There were no significant statistical interactions involving the year factor. Statistical differences in the treatment and rate factors and their interaction were due to variation between the plots prior to the experiment. In April of 1982, a few of the kernels of the original inoculum on the soil surface still contained viable fungus when plated on PDA media, but none of the new thistle shoots were infected with Sclerotinia. The sclerotia remained viable and disease symptoms first appeared in July. By the end of August, the percentage of dead Canada thistle shoots ranged from 26 to 56%, in the 3125 kernels/m^2 rate plots, which was significantly different from the control plots that ranged from 1 to 11% (Table 6,7). The 400 kernels/m^2 rate plots averaged 22% but the 50 kernels/m^2 rate plots were not significantly different from the control plots. By correlation analysis, there was no correlation between the percentage of dead shoots in November 1981 and % kill in August 1982, in the plots treated at either the 3125 kernels/m^2 (r^2 = .0042) or 400 kernels/m^2 (r^2 = .2848) rate.

Table 8. Statistical analysis of thistle density[a] in 1981 and 1982, after *Sclerotinia sclerotiorum* treatment in October 1981.

Source of variation	df	Mean square[b]
2x2x3 factorial ANOV:		
Treatment[c]	1	.0705*
Year	1	.0934*
Rate	2	.1227**
Treatment x year	1	.0012
Treatment x rate	2	.0732*
Year x rate	2	.0093
Treatment x year x rate	2	.0005
Non-factorial partition:		
Among 1981	5	.0572**
Error	36	.0152

[a]Logarithmic transformation of thistle densities. df = degrees of freedom.
[b]* = significant, P = 0.05; ** = significant, P = 0.01.
[c]Treatment = control or *S. sclerotiorum* treatment.

1982 Field Trials

These studies were conducted with *S. sclerotiorum* isolate 81-162-01, and investigated the rate of inoculum application, soil incorporation of inoculum, and timing of application on disease development.

Timing of Inoculum Application

During application, the inoculum often rolled down the lower leaves into the leaf axils. Inoculum particles trapped in this manner almost always infected the axil area. Wilting symptoms appeared 7 to 10 days after field inoculation.

Young Canada thistle plants inoculated in May 1982 became infected with S. sclerotiorum, despite four days with minimum air temperatures below 0° C. However, plots inoculated in June had the highest percentage of dead Canada thistle shoots by the end of the growing season (August 20, 1982) (Figure 3). At the 3125 kernels/m^2 rate of application, the June inoculated plots had an average of 73% shoots dead while averages for the May and July inoculated plots were 59 and 52%, respectively (Table 9). The 313 kernels/m^2 rate of application had lower rates of kill (Table 10). The June inoculated plots averaged 30% dead thistle shoots while the May and July inoculated plots averaged 8% and 16%, respectively (Table 9). The control plots were significantly less than any of the treated plots, and averaged 2%.

The June treatment was significantly higher than the May or July treatments. A quadratic equation was fitted to the data of the S. sclerotiorum treated levels of the date factor (rates combined). On the basis of this equation, the optimal time to apply the inoculum to achieve maximum thistle shoot kill was June 13 (Figure 4).

Very little, if any, thistle shoot regrowth was observed in the inoculated plots during the 1982 growing season. Eight weeks after inoculation, the fungus was found to extend at least 25 cm into the Canada thistle

Figure 3. The percentage of dead thistle shoots over time in field plots inoculated with Sclerotinia sclerotiorum isolate 81-162-01 at the 3125 kernels/m^2 rate in May, June or July.

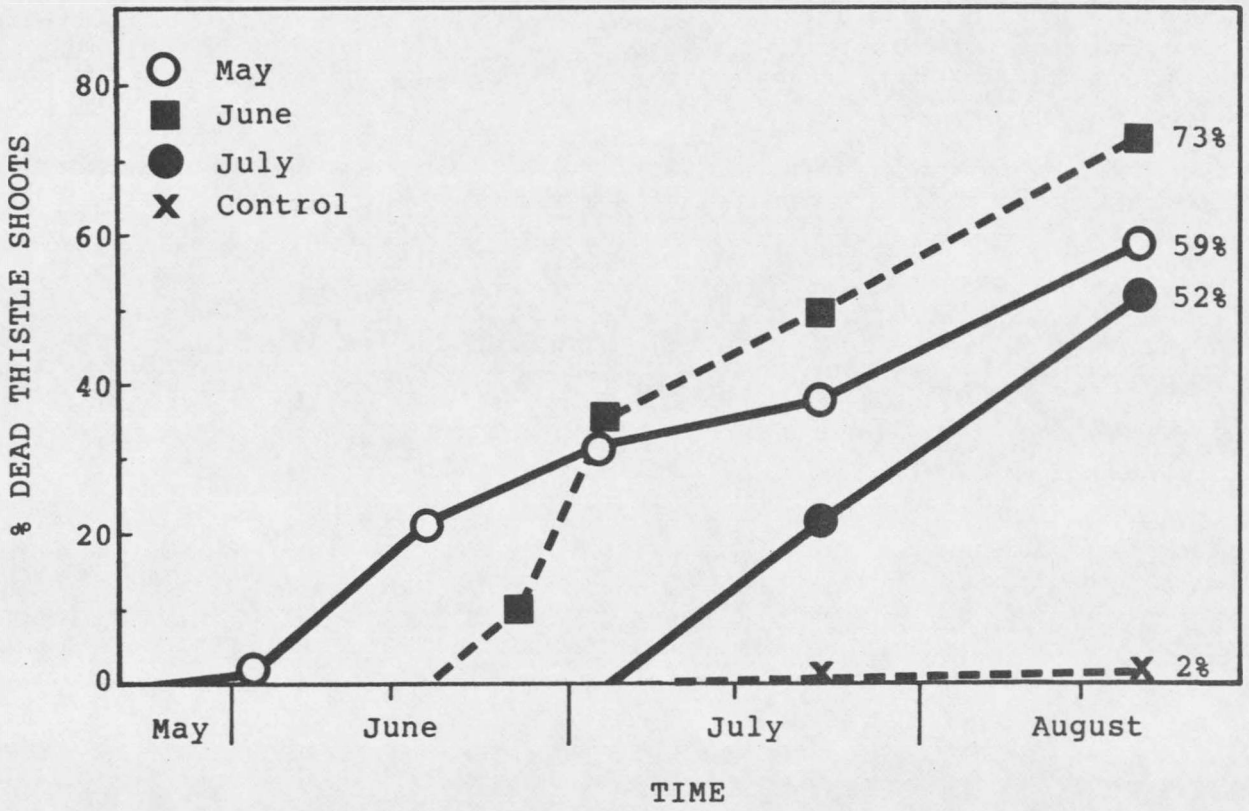


Table 9. The effect of time of *Sclerotinia sclerotiorum* treatment and rate of application on thistle mortality and thistle density[a].

Rate[b] k/m^2	Time of Application	% dead shoots August 1982		Change in thistle density (1983 density ÷ 1982 density)[c]	
		Treated	Control	Treated	Control
3125	May 24	59 ± 12 (47-75)	2 ± 1 (0-3)	.73 (.46-.89)	1.22 (1.02-1.49)
	June 18	73 ± 12 (61-88)	3 ± 2 (0-5)	.90 (.29-1.75)	1.81 (1.38-2.88)
	July 4	52 ± 6 (47-59)	3 ± 1 (2-5)	.92 (.78-1.16)	1.34 (1.29-1.40)
313	May 24	8 ± 4 (3-13)	1 ± 1 (0-2)	1.36 (.86-1.79)	1.13 (.98-1.29)
	June 18	30 ± 14 (17-46)	2 ± 2 (0-4)	1.21 (.56-1.58)	1.33 (.91-2.02)
	July	16 ± 13 (5-34)	0 ± 1 (0-1)	1.35 (.78-1.71)	1.69 (1.04-3.00)

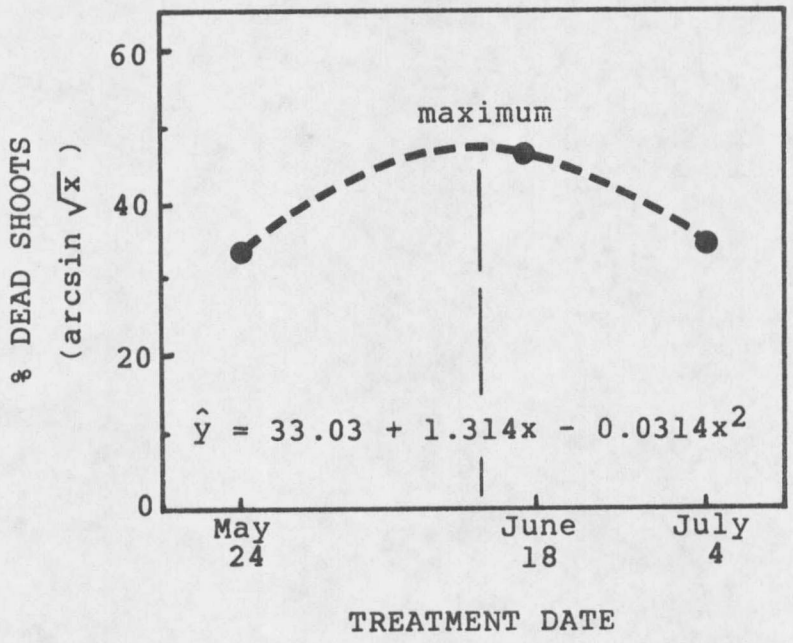
[a]Mean ± standard deviation, with range in parenthesis.
[b]Dried inoculum of isolate 81-162-01 was applied. k = kernels
[c]*Sclerotinia* infected thistles were included in the thistle densities. Thistle densities ranged from 19 to 85 shoots/m^2 in 1982.

Table 10. Statistical analysis of % dead thistle shoots[a], as affected by time of *Sclerotinia sclerotiorum* treatment and rate of application.

Source of variation	df	Mean square[b]
2x2x3 factorial ANOV:		
Blocks	3	71.4
Treatment[c]	1	12130. **
Rate	1	3179. **
Date	2	277.3**
Treatment x rate	1	1804. **
Treatment x date	2	146.5*
Rate x date	2	23.6
Treatment x rate x date	2	70.8
Non-factorial partition:		
Among controls	5	38.7
May 313 rate, treated vs control	1	300.1**
June 313 rate, treated vs control	1	1332.3**
July 313 rate, treated vs control	1	846.3**
Error	33	32.2
2x3 factorial ANOV (excluding controls):		
Rate	1	4885.9**
Date	2	411.6**
Linear	1	43.6
Quadratic	1	779.6**
Rate x date	2	58.6
Error	18	52.6

[a]Arcsine transformation of % dead shoots. df = degrees of freedom.
[b]* = significant, P = 0.05; ** = significant, P = 0.01.
[c]Treatment = control or *S. sclerotiorum* treatment.

Figure 4. Graphic representation of the data of the treated levels of the date factor from the 2x3 factorial ANOV (Table 10) fitted to a quadratic equation.



root system. Two other weeds, field pennycress (Thlaspi arvense) and prickly lettuce (Lactuca scariola) growing in the field plots were sometimes attacked and killed. Spring wheat in the field plots was not affected.

From 1982 to 1983, the thistle shoot density increased an average of 42% in the control plots (Table 9). The thistle density also increased in the 313 kernels/m^2 treated plots but it was not statistically different from the 1982 density (Table 11). In the 3125 kernels/m^2 treated plots, the density decreased significantly from the 1982 density. There was no statistical difference between the dates of application at either of the two rates (Table 11,12). The significant treatment x rate x date interaction is due to variation among the initial control plots. Increased replication is needed to test the effect of time of treatment on the thistle density.

Additionally, it was observed that the S. sclerotiorum isolate 81-162-01 may be less effective against an ecotype of variety arvense (mite), one of the nine ecotypes mapped inside the plot area (Table 13).

In 1983, very few of the thistles showed symptoms of the disease. By July 29, 1983, only 23 thistle shoots or 0.1% of the total number of shoots in the experimental area of 407 m^2 had become infected. By August 25, 1983, 93 shoots or 0.4% of the thistles were infected with

Table 11. Statistical analysis of thistle density[a] in 1982 and 1983, as affected by time of *Sclerotinia sclerotiorum* treatment and rate of application.

Source of variation	df	Mean square[b]
2x2x2x3 factorial ANOV:		
Blocks	3	.0267
Treatment[c]	1	.1394**
Year	1	.0904**
Rate	1	.0346
Date	2	.0049
Treatment x year	1	.1182**
Treatment x rate	1	.1360**
Year x rate	1	.0446
Treatment x date	2	.0018
Year x date	2	.0104
Rate x date	2	.0198
Treatment x year x rate	1	.0866*
Treatment x year x date	2	.0097
Treatment x rate x date	2	.0520*
Year x rate x date	2	.0046
Treatment x year x rate x date	2	.0081
Non-factorial partition:		
Among 1982	11	.0147
Among 1983 control	5	.0110
Control, 1982 versus 1983	1	.2077**
Among 1983 treated, 3125 vs 313 rate	1	.3810**
Among 1983 treated 3125 rate	2	.0023
Among 1983 treated 313 rate	2	.0111
Treated 3125 rate, 1982 versus 1983	1	.0752*
Treated 313 rate, 1982 versus 1983	1	.0535
Error	69	.0145

[a]Logarithmic transformation of thistle densities. df = degrees of freedom.
[b]* = significant, P = 0.05; ** = significant, P = 0.01.
[c]Treatment = control or *S. sclerotiorum* treatment.

Table 12. Further analysis of thistle density in 1982 and 1983, in the Timing of Inoculum Application Experiment[a].

Source of variation	df	Mean square[b]	
		Control	Treated
2x2x3 factorial ANOV:			
Year	1	.2076**	.0009
Rate	1	.0167	.1539**
Date	2	.0034	.0033
Year x rate	1	.0035	.1278**
Year x date	2	.0136	.0066
Rate x date	2	.0624*	.0094
Year x rate x date	2	.0093	.0033
Error	36	.0146	.0154

[a]A 2x2x3 factorial ANOV was done on each level in the treatment factor from the 2x2x2x3 factorial ANOV. df = degrees of freedom.
[b]* = significant, P = 0.05; ** = significant, P = 0.01.

Table 13. Thistle ecotype and variation in the change in thistle density from 1982 to 1983, in individual plots treated with *Sclerotinia sclerotiorum* at the 3125 kernels/m^2 rate.

Date applied	Plot #	% dead shoots August 1982	Change in thistle density[a]
May 24	1	75	.46
	2	55	.83
	3	59	.89
	4	47	.74
June 18	1	76	.52*
	2	88	.29
	3	61	1.75*
	4	68	1.04*
July 4	1	48	1.16*
	2	47	.81
	3	55	.92*
	4	59	.78

[a]Change in thistle density = 1983 density ÷ 1982 density. The starred plots were composed entirely of an ecotype of variety *arvense*. The other plots consisted of ecotypes of varieties *horridum* or *vestitum*, or combinations of ecotypes of *horridum*, *vestitum* and *arvense*.

Sclerotinia. Infected thistles were found in control as well as treated plots, and airborne ascospores were trapped on July 19 and 27, 1983. In an adjacent area at this site, treated in July 1983 with grain kernel inoculum from 150 different Sclerotinia isolates, high rates of infection and subsequent shoot death occurred. During the winter of 1982, the lowest air temperature was -21° C. Climatological information for 1983 is summarized in Table 23 (Appendix).

Soil Incorporation of Inoculum

Incorporation of the inoculum into the soil did increase the percentage of dead Canada thistle shoots over soil surface application, but the difference was not statistically significant (Table 14,15). The soil incorporation treatment averaged 57%, and the soil surface treatment averaged 49% dead thistle shoots. Both treatments were, however, significantly different from the control plots that averaged 2% dead shoots.

In 1983, the thistle density increased an average of 105% in the control plots (Table 14,16). In the treated plots, the thistle density increased 6% in the soil surface treatment but decreased 51% in the soil incorporation treatment. In the soil surface treated plots, the 1983 density was not significantly different from the 1982 density (Table 16).

Table 14. Canada thistle mortality, and change in thistle density following surface or soil incorporation application of *Sclerotinia sclerotiorum* inoculum[a].

Application[b]	% dead shoots August 1982		Change in thistle density (1983 density ÷ 1982 density)[c]	
	Treated	Control	Treated	Control
Soil surface	49 ± 6 (42-56)	2 ± 3 (1-6)	1.06 (.55-1.80)	2.29 (1.72-2.83)
Soil incorporation	57 ± 17 (32-71)	2 ± 3 (0-6)	.49 (.22-.86)	1.82 (1.43-2.13)

[a]Mean ± standard deviation, with range in parentheses.
[b]Dried inoculum of isolate 81-162-01 was applied.
[c]*Sclerotinia* infected thistles were included in the thistle densities. Thistle densities ranged from 30 to 51 shoots/m^2 in 1982.

Table 15. Statistical analysis of % dead shoots[a] following surface or soil incorporation application of *Sclerotinia sclerotiorum* inoculum.

Source of variation	df	Mean square[b]
2x2 factorial ANOV:		
Treatment[c]	1	6094. **
Application	1	14.6
Treatment x application	1	30.2
Error	12	40.7

[a]Arcsine transformation of % dead shoots. df = degrees of freedom.
[b]** = significant, P = 0.01.
[c]Treatment = control or *S. sclerotiorum* treatment.

Table 16. Statistical analysis of thistle density[a] in 1982 and 1983, following surface or soil incorporation application of *Sclerotinia sclerotiorum* inoculum.

Source of variation	df	Mean square[b]
2x2x2 factorial ANOV:		
Treatment[c]	1	.6474**
Year	1	.0269
Application	1	.0175
Treatment x year	1	.4863**
Treatment x application	1	.1388**
Year x application	1	.1002**
Treatment x year x application	1	.0314
Non-factorial partition:		
Among 1982	3	.0139
Among 1983 control	1	.0026
Control, 1982 versus 1983	1	.3710**
Surface treated, 1982 versus 1983	1	.0004
Soil incorp. treated, 1982 vs 1983	1	.2638**
Error	24	.0131

[a]Logarithmic transformation of thistle densities. df = degrees of freedom.
[b]** = significant, P = 0.01.
[c]Treatment = control or *S. sclerotiorum* treatment.

Statewide Research Trials

At all eight of the field research sites where Canada thistle was inoculated with *S. sclerotiorum*, the thistles became infected with the fungus. Data were collected at five of these research sites. Average percentages of dead Canada thistle shoots ranged from 20 to 80% (Table 17). These figures partially reflect different inoculation and data collection times. At all five of the sites, the percentage of dead shoots was significantly higher in the treated plots than in the control plots. At the Benchland site, two of the inoculated plots had 90 and 92% dead thistle shoots. These two plots also had very high thistle densities of 155 and 146 shoots/m^2 versus 59 shoots/m^2 in the two plots that had 61 and 76% dead shoots. Climatological information for 1982 is listed in Table 22 (Appendix).

Thistle density data for 1983 were collected only at the Benchland and Corvallis sites. At the pasture site at Benchland the thistle density was reduced 95% in the treated plots, from 105 shoots/m^2 in 1982 to 4 shoots/m^2 in 1983 (Table 18). This difference was statistically significant (Table 19). The thistle density in the control plots was reduced 32%. Grasses replaced the thistles in the treated plots. *Sclerotinia* symptoms were observed within the control plots in July of 1983.

Table 17. Canada thistle mortality in the Statewide Research Trials in 1982 after *Sclerotinia sclerotiorum* treatment.

Location	Date of application[a]	Date data collected	% dead shoots[b]	
			Treated	Control
Havre	June 2	July 7	44 ± 12** (29-59)	4 ± 6 (0-12)
Moccasin	June 14	August 28	79 ± 8 * (73-90)	50 ± 22 (27-70)
Benchland	June 16	August 28	80 ± 14** (61-92)	31 ± 8[c] (22-42)
Corvallis	June 7	July 7	61 ± 13** (46-76)	5 ± 4 (0-10)
Huntley	July 2	August 25	19 ± 3** (15-21)	2 ± 2[d] (0-4)

[a]Dried inoculum of isolate 81-162-01 was applied.
[b]Mean ± standard deviation, with range in parentheses. At each location, the % dead shoots was transformed to arcsin $\sqrt{x}$, and the treated and control values analysed by the Student's t test. * = significant, P = 0.05; ** = significant, P = 0.01.
[c]*Sclerotinia* moved 0.3 m into neighbouring control plots.
[d]Huntley site was dry and hot following inoculum application, receiving only 38 mm of precipitation from July 2 to August 25, 1982 (Table 25).

Table 18. The effect of *Sclerotinia sclerotiorum* treatment on thistle density at the Statewide Research Trial sites at Benchland and Corvallis[a].

Location	Thistle density 1982		Thistle density 1983		Change in thistle density[b] (1983 density ÷ 1982 density)	
	Treated	Control	Treated	Control	Treated	Control
Benchland	105 ± 53 (59-155)	93 ± 47 (62-162)	4 ± 3 (1-8)	60 ± 18 (46-86)	.05 (.01-.14)	.68 (.53-.77)
Corvallis	15 ± 3 (10-17)	14 ± 4 (10-19)	2 ± 1 (1-3)	5 ± 1 (3-6)	.14 (.07-.30)	.34 (.25-.50)

[a]Mean ± standard deviation, with range in parentheses. Thistle density in shoots/m^2.
[b]*Sclerotinia* infected thistles were included in the thistle densities.

Table 19. Statistical analysis of thistle density[a] in 1982 and 1983 at two Statewide Research Trial sites after *Sclerotinia sclerotiorum* treatment.

Source of variation	df	Mean square[b]	
		Benchland	Corvallis
2x2 factorial ANOV:			
Treatment[c]	1	1.254**	.086*
Year	1	2.377**	1.432**
Treatment x year	1	1.443**	.108*
Non-factorial partition:			
Among 1982	1	.003	.001
Among controls, 1982 vs 1983	1	.058	.377**
Among treated, 1982 vs 1983	1	3.762**	1.163**
Error	12	.046	.014

[a]Logarithmic transformation of thistle densities. df = degrees of freedom.
[b]* = significant, P = 0.05; ** = significant, P = 0.01.
[c]Treatment = control or *S. sclerotiorum* treatment.

The 1983 thistle densities at the Corvallis site were consistently low for all of the plots (Table 18,19). Reasons for the reduction are unclear.

Sclerotinia infection was observed by June in the 1983 field trial at Huntley inoculated with S. sclerotiorum on May 19. By June 20, the plots treated with S. sclerotiorum at the 3125 kernels/m^2 rate averaged 26% dead thistle shoots which was significantly different from the control plots that averaged 3% and the 313 kernels/m^2 rate plots that averaged 7% (Table 20,21). The 313 kernels/m^2 rate was not significantly different from the controls. June through August was dry and hot with only 83 mm of precipitation (Table 23 in Appendix). The month of June received less than two-fifths of the normal projected precipitation and established ornamental shrubs next to the plot site died from drought stress. The thistles in the plot area were also suffering from drought stress. By August 22, there was no significant difference among control, and 3125 kernels/m^2 and 313 kernels/m^2 rate treatments (Table 21).

Mycelial Spread

Spread of the fungus from the place of inoculation during one growing season was limited in the S. sclerotiorum mycelial spread plots put out in 1982 in Bozeman and at the seven field research sites. The

Table 20. Canada thistle mortality in the 1983 Statewide Research Trial at Huntley, following *Sclerotinia sclerotiorum* treatment.

Application rate[b]	% dead shoots[a]	
	June 20	August 22[c]
3125 kernels/m^2	26 ± 8 (16-35)	85 ± 20 (54-95)
313 kernels/m^2	7 ± 3 (4-9)	73 ± 17 (59-93)
Control	3 ± 3 (0-6)	57 ± 22 (41-88)

[a]Mean ± standard deviation, with range in parentheses.
[b]Fresh inoculum of *S. sclerotiorum* isolate 81-162-01 was applied at two rates on May 19, 1983.
[c]June through August was hot and dry, with only 83 mm total precipitation (Table 26).

Table 21. Statistical analysis of % dead shoots[a] in June and August at the 1983 Statewide Research Trial at Huntley.

Source of variation	df	Mean square[b]	
		June 20	August 22
1-way ANOVA:			
Rate	2	561.3**	383.4
Error	9	33.8	184.0

[a]Arcsine transformation of % dead shoots. df = degrees of freedom.
[b]** = significant, P = 0.01.

fungus moved at most 20 to 30 cm from the inoculated plant and often only the inoculated thistle plant died. In the 1982 Field Trials, the plot borders were well-defined in 1982, with the fungus again moving 20 to 30 cm at most into any neighboring control plot.

DISCUSSION

Selection

Of the six pathogens found to occur on Canada thistle in Montana, *Sclerotinia* appears to have the most potential as a biological control agent of Canada thistle. This fungus is endemic, lethal to Canada thistle and will infect the root system. *Sclerotinia* symptoms appear within 5 to 10 days after inoculation, and all Canada thistle growth stages are susceptible. It is a soilborne pathogen capable of growing under a wide range of environmental conditions including temperature and pH, and produces no asexual stage. In addition, *S. sclerotiorum* may be tolerant of several herbicides (84,85). It is easy and fast to grow in culture, and, according to Kreitlow (59), the dried grain inoculum can be stored up to 2 years. Lastly, its predominantly haploid genetic system make it a good candidate for genetic studies to select and develop improved strains.

Sclerotinia as a Biocontrol Agent

Under natural conditions in Montana, *Sclerotinia* disease symptoms were usually not observed until late June to July, which may be too late in the season to stop

seed production for that year. The fungus also has difficulty in spreading over an area infested with Canada thistle under the environmental conditions found in Montana. By applying laboratory produced inoculum earlier in the growing season, these limiting factors can be reduced or eliminated.

Sclerotinia killed Canada thistle shoots in greenhouse studies. Differences in virulence were found between the ten Sclerotinia isolates tested on Canada thistle with the isolates originally from this weed being much more pathogenic on thistle than the isolates from safflower or bean. In addition, a thistle isolate from an area previously cropped to faba beans and iris, and a S. minor isolate from Canada thistle were less pathogenic than the other Canada thistle isolates. Canada thistle may have some levels of resistance to the Sclerotinia isolates, since some of the inoculated thistle plants developed only small lesions at the site of inoculation. Price and Colhoun (80) in their host range studies also occasionally noted a slight pathogenic reaction of small discrete stem lesions that did not affect plant vigor.

Sclerotinia sclerotiorum killed Canada thistle shoots in the field under adverse conditions. In the Fall 1981 Trials, the inoculum was subjected to snow and cold temperatures. Dijkstra (35) found frost injury in red clover enhanced the spread of S. trifoliorum. In the

trials, the fungus did not have enough time to infect the roots before winter and the density of Canada thistle shoots that sprouted from the root system the following spring was not reduced. The Fall 1981 Trials did not show disease symptoms in the spring of 1982, as very few of the inoculum kernels still contained viable Sclerotinia mycelium and the fungus did not overwinter in the thistle root system. Similarly, Cook et al. (30) found S. sclerotiorum did not survive as mycelium in plant residue but could overwinter in Nebraska as mycelium in 2% of the bean seed left on the soil surface from the previous summer, or as sclerotia in the soil. Disease symptoms did however, appear in the treated plots in July and August of 1982. Infection was probably from mycelial or carpogenic germination of the initial inoculum sclerotia.

Although Sclerotina symptoms do not appear in Montana until late June under natural conditions, young thistle plants could be infected with the disease in field inoculated plots in May. All of the field tests were conducted with one isolate of S. sclerotiorum (81-162-01) and with a single application of the inoculum to each individual plot. This isolate was effective under different environmental conditions as demonstrated in the Statewide Research Trials, and killed from 19 to 80% of the thistle shoots in the treated plots.

Inoculation in June gave the highest level of control as shown in the 1982 Field Trials. There was little, if any, thistle shoot regrowth in the inoculated plots over the growing season, limiting the amount of food reserves directed into the root system. In addition, the fungus invaded the root system, damaged the roots and reduced the thistle density by 8 to 95% the following year. Additional studies, with increased replication, are needed to study the relationship between the degree of thistle mortality and reduction in thistle density the year following treatment.

Recurrence

The percentage of *S. sclerotiorum* infected thistles was very low the year following spring or summer treatment, at all three of the research trial sites where data was collected in 1983. At the site of the 1982 Field Trials, only 0.4% of the thistle shoots had *Sclerotinia* symptoms as of August 27, 1983. The lack of symptom development could be due to two processes: sclerotia destruction and poor ascospore infection. It is not known if it is possible for the fungus to overwinter as mycelium inside the Canada thistle root system.

Sclerotia destruction by environmental factors and soil microflora would diminish the inoculum available for

infection. Better sclerotia survival may account for the higher percentage of Sclerotinia diseased thistles observed in 1982 in the Fall 1981 Field Trials. In these trials the inoculum had been mixed into the top layer of the soil.

Despite some airborne ascospores being trapped at the site of the 1982 Field Trials during 1983, the percentage of infected thistles was very low. The fact that diseased thistles were found in both treated and control plots lends support to ascospore infection. The 48 to 72 hour requirement of continuous free water combined with the need of an exogenous food source for ascospore infection, may be limiting infection under natural conditions in Montana. Frosts occur until late May to early June. By July, the air temperatures exceed 25^{o} C and precipitation is reduced. Much of the summer rainfall is in the form of periodic thundershowers. Furthermore, the thistles have very little senescent or necrotic tissue before July. This exogenous food source is needed by the ascospore before it can infect the host.

Infection under natural conditions and in the field experiments was never observed above the lower stem to crown region of the thistle shoot. This was partially influenced by the surrounding vegetation and its effect on the microclimate. Grogan and Abawi (40) postulated that the free water required for lesion initiation and

expansion may be needed to allow diffusion of toxins and enzymes needed for pathogenicity.

Secondary Spread

After the initial infection, the Canada thistle root system plays an important part in the secondary spread of this disease and results in the disease pattern of small patches of dead thistles observed under natural conditions. Another type of disease pattern was observed by Bodine (21) over a two year period in Colorado. A natural Sclerotinia infection of an undisturbed infestation of Canada thistle resulted in the death of the majority of the thistle shoots in the center of the patch. The thistle was able to outgrow the fungus and maintain an outer leading edge of healthy Canada thistle shoots. A similar pattern was observed at one location in Montana during the Thistle Disease Surveys.

Secondary spread through the roots was also observed in the field studies, where in one growing season, the fungus sometimes moved 20 to 30 cm into neighboring plots. At the site of the 1982 Field Trials, the mycelial spread could have been restricted by the fall 1981 cultivation that would have disturbed the interconnecting root system. But providing enough Sclerotinia inoculum is applied, high rates of thistle kill still occurred at this site. Soil incorporation of

the inoculum resulted in higher levels of thistle control than surface inoculum application. Only two of the five Statewide Research Trials where data were collected, Havre and Benchland, were not cultivated prior to the experiments. The highest level of thistle control of all the field studies was achieved at the Benchland site, where the thistle density was reduced by 95% the year following inoculation. At Benchland, S. sclerotiorum did spread 30 cm into the neighboring control plots, and a high thistle density could have enhanced the disease spread by increasing the moisture conditions in the microclimate. On an uncultivated site, such as pasture, a lower rate of inoculum application might be possible.

Future Considerations

Two aspects, efficient economical inoculum production and host range reduction, need to be resolved before Sclerotinia sclerotiorum can be used as a biological control agent of Canada thistle.

The dried inoculum used to date, is easy to handle and can be stored at 0 to 5° C for 24 months with no appreciable reduction in pathogenicity (59). However, the infected kernel must be in contact with the thistle for the plant to become infected. A smaller inoculum particle size would increase thistle coverage, and reduce the quantity of inoculum required. In addition,

microbial action on the kernel inoculum may be reducing the inoculum's potential viability. By visual observations, many of the infected kernels applied in the field plots did not produce mycelium. In fact, Sclerotinia mycelium has been reported to be shortlived or of low saprophytic ability in soil (30,64,75). Combining a smaller inoculum particle size, protection against harmful soil microorganisms and the optimal nutrients required for infection, much lower inoculum application rates might be feasible.

Considering host range, wheat, barley, oats, and corn are not affected by S. sclerotiorum, S. trifoliorum, or S. minor, and cereal grains are often used in a rotation program to avoid buildup of Sclerotinia in the soil. Still, some of the other hosts tested in the Host Range Studies were susceptible to our Sclerotinia isolates, including safflower, sunflower, beans and lentils. Sclerotinia spp. will also infect many weeds, including diffuse knapweed (Centaurea diffusa) (114). In Montana, Sclerotinia is rarely found in susceptible varieties of safflower, bean and sunflower outside of intensively cropped irrigated areas.

Bodine (21) and Morgan (70) working in areas with a more varied agriculture rejected using Sclerotinia spp. as biological weed control agents because of the susceptibility of non-target hosts. Very little work has

been done to efficiently select or genetically alter Sclerotinia for restricted host range or other desirable traits. Commercial development of a wild type is perhaps an unreasonable expectation. Yet the economic gains made from the domestication of other fungi such as Penicillium, Aspergillis, and Saccharomyces, are proof of the genetic changes possible from simple selection techniques. Modern techniques for genetic manipulation should greatly reduce the effort required for future fungal domestication projects.

Compared to other plant pathogens, Sclerotinia is easy to identify, culture, and store. This has resulted in much knowledge of its biology and control. Several of the studies done for this project and projects of other research groups indicate that additional research into the overwintering, spread, host range, and genetics should be highly fruitful. Researchers are now attempting to take advantage of the present data base to make Sclerotinia one of the first plant pathogens to be used in a manageable biocontrol system for weeds.

SUMMARY

1. Six pathogens were found to occur on Canada thistle in Montana. These included Puccinia punctiformis and pathogens belonging to the following genera: Septoria, Fusarium, Sclerotinia, Pseudomonas and Alternaria.

2. Sclerotinia was selected for further research primarily because of its lethality, quick development of disease symptoms, ability to infect the root system and ease to culture and store. The fungus has the added advantages of fast field diagnosis and possibly herbicide tolerance. In addition, its predominantly haploid genetic system make it a good candidate for genetic studies to select and develop improved strains.

3. Sclerotinia diseased Canada thistles were found to occur in small patches up to 1.5 x 1.2 m wide from late June to September throughout most of Montana. The short growing season and dry summer results in late and limited ascospore infection under natural conditions.

4. Sclerotinia caused a lethal wilt of Canada thistle in both greenhouse and field studies. Using mycelial

inoculum of a single isolate of S. sclerotiorum for all of the Canada thistle field studies, the fungus was capable of infecting in the spring, summer, and fall, and any growth stage of the thistle. Inoculation in June resulted in 73% kill of the thistle shoots within 9 weeks in the 1982 Field Trials. In Statewide Research Trials the fungus killed 19 to 80% of the thistle shoots in the treated plots. The fungus invaded the root system and reduced the thistle density 8 to 95% the following year.

5. Soil incorporation of the S. sclerotiorum inoculum was more effective than surface application, and reduced the thistle density by 51% the following year.
6. The highest level of thistle control in all of the field trials was on uncultivated land. At the pasture site at Benchland, the thistle density in the treated plots was reduced 95% the following year.
7. In the plot areas treated during 1982, the percentage of Sclerotinia infected thistles was very low in 1983. Only 0.4% of the thistle shoots had disease symptoms at the site of the 1982 Field Trials on August 27, 1983. The lack of symptom development could be due to sclerotia destruction or poor ascospore infection. Plots treated with fresh kernel

inoculum during 1983 at the site of the 1982 Field Trials, did have high rates of shoot kill. Higher levels of Sclerotinia incidence were observed at the 1981 Fall Field Trials during 1982, where the inoculum had been applied October 21, 1981 and soil incorporated.

8. Wheat, barley, oats, corn and potato were not susceptible to Sclerotinia isolates collected during the Canada Thistle Disease Survey. Other hosts, including safflower, sunflower, beans and lentils were susceptible. Differences in virulence were observed among the isolates.

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APPENDIX

Table 22. 1982 climatological information at the field trial locations[a].

Site	Precipitation (mm)				Average temperature (°C)							
					May		Jun		Jul		Aug	
	May	Jun	Jul	Aug	max	min	max	min	max	min	max	min
Havre	140	25	41	38	18	3	25	8	29	11	31	10
Moccasin[b]	90	74	35	18	14	2	21	8	26	11	28	10
Corvallis	28	41	35	25	19	1	24	6	27	10	29	9
Huntley	58	88	37	5	18	3	22	8	28	11	31	11
Bozeman 1[c]	67	90	38	20	16	2	21	7	26	9	28	9
Bozeman 2[d]	73	101	39	26	16	3	21	8	26	11	29	11

[a]References 26 and 74.
[b]The Benchland site is 5 miles west of Moccasin.
[c]Arthur Post Agricultural Research Center.
[d]Montana State University. The Fort Ellis Station is 4 miles east of the university.

Table 23. 1983 precipitation and normal precipitation (mm) at the field trial locations[a].

Site	1983				Normal[b]			
	May	Jun	Jul	Aug	May	Jun	Jul	Aug
Moccasin[c]	47	61	71	29	66	84	41	39
Corvallis	39	28	62	41	44	40	19	22
Huntley	52	24	37	22	54	62	24	25
Bozeman 2[d]	62	73	66	36	66	83	30	35

[a]References 26 and 74.
[b]Averaged from: Moccasin (1909-1982), Corvallis (1965-1982), Huntley (1909-1982), Bozeman 2 (1940-1969).
[c]The Benchland site is 5 miles west of Moccasin.
[d]Montana State University. The Fort Ellis Station is 4 miles east of the university.